

EXHIBIT 4

EXHIBIT 4**Claim Charts for '540 Patent: Aria Harmony Prenatal Test™**

Asserted Claims of U.S. Patent No. 6258,540	Aria's "Harmony Prenatal Test™" utilizing Aria's DANSR/FORTE technology
<p>1. A method for detecting a paternally inherited nucleic acid of fetal origin performed on a maternal serum or plasma sample from a pregnant female, which method comprises</p>	<p>"The Harmony Prenatal Test utilizes new technology which couples innovative biochemistry, DANSR™, and a proprietary algorithm, FORTE™, to efficiently analyze patients' blood samples." [Aria Diagnostics Press Release 2/6/2012.]</p> <p>"Objective</p> <p>To develop a novel biochemical assay and algorithm for the prenatal evaluation of risk for fetal trisomy 21 and 18 using cell-free DNA obtained from maternal blood.</p> <p>Study Design</p> <p>We assayed cfDNA from a training set and a blinded validation set of pregnant women, comprising 250 disomy, 72 trisomy 21 (T21), and 16 trisomy 18 (T18) pregnancies. We used Digital ANalysis of Selected Regions (DANSR) in combination with a novel algorithm, Fetal-fraction Optimized Risk of Trisomy Evaluation (FORTE) to determine trisomy risk for each subject." [American Journal of Obstetrics and Gynecology, Sparks et al. ("Aria AJOG Publication"), Abstract.]</p> <p>"In this study, we extend DANSR to assay simultaneously polymorphic and non-polymorphic loci in a single reaction, enabling estimation of chromosome proportion and fetal fraction." [Aria AJOG Publication at 5.]</p> <p>"8mL blood per subject was collected into a Cell-free DNA tube (Streck) and stored at room temperature for up to 3 days. Plasma was isolated from blood via double centrifugation and stored at -20C for up to a year. cfDNA was isolated from plasma using Viral NA DNA purification beads (Dyna), biotinylated, immobilized on MyOne C1 streptavidin beads (Dyna), and annealed with the multiplexed DANSR oligonucleotide pool. [Aria AJOG Publication at 7.]</p>

<p>amplifying a paternally inherited nucleic acid from the serum or plasma sample and</p>	<p>“DANSR assay</p> <p>We designed DANSR assays against loci in the human genome as previously described. To assess chromosome proportion, we designed assays against 576 non-polymorphic loci on each of chr18 and chr21, where each assay consisted of three locus specific oligonucleotides: a left oligo with a 5’ universal amplification tail, a 5’ phosphorylated middle oligo, and a 5’ phosphorylated right oligo with a 3’ universal amplification tail. To assess fetal fraction, we designed assays against a set of 192 SNP-containing loci on chr1-12, where two middle oligos, differing by one base, were used to query each SNP. SNPs were optimized for minor allele frequency in the HapMap 3 dataset (http://hapmap.ncbi.nlm.nih.gov/). Oligonucleotides were synthesized by IDT and pooled together to create a single multiplexed DANSR assay pool.”</p> <p>[Aria AJOG Publication at 6.]</p> <p>“8mL blood per subject was collected into a Cell-free DNA tube (Streck) and stored at room temperature for up to 3 days. Plasma was isolated from blood via double centrifugation and stored at -20C for up to a year. cfDNA was isolated from plasma using Viral NA DNA purification beads (Dynal), biotinylated, immobilized on MyOne C1 streptavidin beads (Dynal), and annealed with the multiplexed DANSR oligonucleotide pool. Appropriately hybridized oligonucleotides were catenated with Taq ligase, eluted from the cfDNA, and amplified using universal PCR primers. PCR product from 96 independent samples was pooled and used as template for cluster amplification on a single lane of a TruSeq v2 SR flow slide (Illumina). The slide was processed on an Illumina HiSeq 2000 to produce a 56 base locus-specific sequence and a 7 base sample tag sequence from an average of 1.18 million (M) clusters/sample. Locus specific reads were compared to expected locus sequences. An average of 1.15M (97%) reads had fewer than 3 mismatches with expected locus sequences, resulting in an average of 854 reads/locus/sample.</p> <p>[Aria AJOG Publication at 7.]</p>
<p>detecting the presence of a paternally inherited nucleic acid of fetal origin in the sample.</p>	<p>“DANSR assay</p> <p>We designed DANSR assays against loci in the human genome as previously described. To assess chromosome proportion, we designed assays against 576 non-polymorphic loci on each of chr18 and chr21, where each assay consisted of three locus specific oligonucleotides: a left oligo with a 5’ universal amplification tail, a 5’ phosphorylated middle oligo, and a 5’ phosphorylated right oligo with a 3’ universal amplification tail. To assess fetal fraction, we designed assays against a set of 192 SNP-containing loci on chr1-12,</p>

where two middle oligos, differing by one base, were used to query each SNP. SNPs were optimized for minor allele frequency in the HapMap 3 dataset (<http://hapmap.ncbi.nlm.nih.gov/>). Oligonucleotides were synthesized by IDT and pooled together to create a single multiplexed DANSR assay pool.”
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Analysis of non-polymorphic loci for chromosome proportion

Sequence counts were normalized by systematically removing sample and assay biases. Sequence counts follow a log normal distribution, so biases were estimated using median polish on log transformed counts.²⁵⁻²⁷ A chr21 proportion metric was then computed for each sample as the mean of counts for selected chr21 loci divided by the sum of the mean of counts for selected chr21 loci and the mean of counts for all 576 chr18 loci. A chr18 proportion metric was similarly calculated for each sample. A standard Z test of proportions was used to compute Z Statistics,

$$z_j = \frac{p_j - p_0}{\sqrt{\frac{p_0(1-p_0)}{n_j}}}$$

where p_j is the observed proportion for a given chromosome of interest in a given sample j , p_0 is the expected proportion for the given test chromosome calculated as the median p_j , and n_j is the denominator of the proportion metric. ...”

[Aria AJOG Publication at 7-8.]

“Locus selection using training samples

Sequence count data from the training samples were first normalized as described above and previously. These samples were subsequently analyzed to select 384 of the 576 loci on chr21 and chr18 best able to discriminate T21 and T18 from normal samples. The 384 loci on each chromosome exhibiting the greatest residual difference between normal and trisomy samples were identified using Z Statistics derived from individual loci for the test chromosome and all 576 loci for the comparison chromosome.

	<p>Analysis of polymorphic loci for fetal fraction</p> <p>Informative polymorphic loci were defined as loci where fetal alleles differ from maternal alleles. Because DANSR exhibits allele specificities exceeding 99%, informative loci were readily identified when the fetal allele proportion of a locus was measured to be between 1 and 20%. A maximum likelihood estimate using the binomial distribution was employed to determine the most likely fetal fraction based upon measurements from several informative loci. The results correlate well ($R^2 > 0.99$) with the weighted average approach presented by Chu and colleagues.” [Aria AJOG Publication at 8-9.]</p> <p>“DANSR enables genotyping of individual polymorphic loci which is not possible using current MPSS approaches. DANSR allowed us to develop an integrated assay to assess polymorphic as well as non-polymorphic loci, thereby permitting simultaneous determination of fetal fraction and chromosome proportion. We used fetal fraction information by imposing a QC requirement that each sample have at least 3% fetal DNA, thereby avoiding low confidence calls arising from low proportions of fetal DNA. In addition, we developed the FORTE algorithm to produce a fetal fraction-dependent risk score indicating the odds of a sample being trisomic versus disomic.” [Aria AJOG Publication at 14.]</p> <p><u>“Importance of fetal fraction</u></p> <p>A principal determinant of the accuracy of any cfDNA analysis method is the fraction of fetal cfDNA in the sample. The higher the fraction of fetal cfDNA, the greater the difference in the number of cfDNA fragments originating from trisomic versus disomic chromosomes and hence the easier it is to detect trisomy. The FORTE algorithm explicitly accounts for fetal fraction in calculating trisomy risk.” [Aria AJOG Publication at 16.]</p>
<p>2. The method according to claim 1, wherein the foetal nucleic acid is amplified by the polymerase chain reaction.</p>	<p>“Appropriately hybridized oligonucleotides were catenated with Taq ligase, eluted from the cfDNA, and amplified using universal PCR primers. PCR product from 96 independent samples was pooled and used as template for cluster amplification on a single lane of a TruSeq v2 SR flow slide (Illumina). [Aria AJOG Publication at 7.]</p>

<p>8. The method according to claim 1, wherein the presence of a foetal nucleic acid from a paternally-inherited non-Y chromosome is detected.</p>	<p>“DANSR assay</p> <p>We designed DANSR assays against loci in the human genome as previously described. To assess chromosome proportion, we designed assays against 576 non-polymorphic loci on each of chr18 and chr21, where each assay consisted of three locus specific oligonucleotides: a left oligo with a 5’ universal amplification tail, a 5’ phosphorylated middle oligo, and a 5’ phosphorylated right oligo with a 3’ universal amplification tail. To assess fetal fraction, we designed assays against a set of 192 SNP-containing loci on chr1-12, where two middle oligos, differing by one base, were used to query each SNP. SNPs were optimized for minor allele frequency in the HapMap 3 dataset (http://hapmap.ncbi.nlm.nih.gov/). Oligonucleotides were synthesized by IDT and pooled together to create a single multiplexed DANSR assay pool.”</p> <p>[Aria AJOG Publication at 6.]</p> <p>“Analysis of polymorphic loci for fetal fraction</p> <p>Informative polymorphic loci were defined as loci where fetal alleles differ from maternal alleles. Because DANSR exhibits allele specificities exceeding 99%, informative loci were readily identified when the fetal allele proportion of a locus was measured to be between 1 and 20%. A maximum likelihood estimate using the binomial distribution was employed to determine the most likely fetal fraction based upon measurements from several informative loci.”</p> <p>[Aria AJOG Publication at 8.]</p>
<p>19. The method according to claim 1, wherein the sample contains foetal DNA at a fractional concentration of total DNA of at least about 0.14%, without subjecting it to a foetal DNA enrichment step.</p>	<p>“Chromosome proportion Z Statistics in training set</p> <p>In order to select loci to be used for aneuploidy detection, we evaluated a set of subjects whose aneuploidy status was known. This training set consisted of 127 normal, 36 T21, and 8 T18 pregnancies. Six normal, 1 T18, and 1 T21 samples (8/171, or 5%) did not meet QC criteria (low count, fetal fraction <3%, and/or evidence from SNPs of a non-singleton pregnancy) and were removed from the dataset.”</p> <p>[Aria AJOG Publication at 11.]</p>

<p>20. The method according to claim 19, wherein the fractional concentration of foetal DNA is at least about 0.39%.</p>	<p>“Chromosome proportion Z Statistics in training set</p> <p>In order to select loci to be used for aneuploidy detection, we evaluated a set of subjects whose aneuploidy status was known. This training set consisted of 127 normal, 36 T21, and 8 T18 pregnancies. Six normal, 1 T18, and 1 T21 samples (8/171, or 5%) did not meet QC criteria (low count, fetal fraction <3%, and/or evidence from SNPs of a non-singleton pregnancy) and were removed from the dataset.”</p> <p>[Aria AJOG Publication at 11.]</p>
<p>21. A method of performing a prenatal diagnosis, which method comprises the steps of:</p> <p>(i) providing a maternal blood sample;</p> <p>(ii) separating the sample into a cellular and a non-cellular fraction;</p> <p>(iii) detecting the presence of a nucleic acid of foetal origin in the non-cellular fraction according to the method of claim 1;</p> <p>(iv) providing a diagnosis based on the presence and/or quantity and/or sequence of the foetal nucleic acid.</p>	<p>“The Harmony Prenatal Test utilizes new technology which couples innovative biochemistry, DANSR™, and a proprietary algorithm, FORTE™, to efficiently analyze patients’ blood samples.”</p> <p>[Aria Diagnostics Press Release 2/6/2012.]</p> <p>“Objective</p> <p>To develop a novel biochemical assay and algorithm for the prenatal evaluation of risk for fetal trisomy 21 and 18 using cell-free DNA obtained from maternal blood.</p> <p>Study Design</p> <p>We assayed cfDNA from a training set and a blinded validation set of pregnant women, comprising 250 disomy, 72 trisomy 21 (T21), and 16 trisomy 18 (T18) pregnancies. We used Digital ANalysis of Selected Regions (DANSR) in combination with a novel algorithm, Fetal-fraction Optimized Risk of Trisomy Evaluation (FORTE) to determine trisomy risk for each subject.”</p> <p>[Aria AJOG Publication, Abstract.]</p> <p>“In this study, we extend DANSR to assay simultaneously polymorphic and non-polymorphic loci in a single reaction, enabling estimation of chromosome proportion and fetal fraction.”</p> <p>[Aria AJOG Publication at 5.]</p> <p>“DANSR assay</p> <p>We designed DANSR assays against loci in the human genome as previously described. To assess chromosome proportion, we designed assays against 576 non-polymorphic loci on each of chr18 and chr21, where each assay consisted of three locus specific oligonucleotides: a left oligo with a 5’ universal amplification tail, a 5’ phosphorylated middle oligo, and a 5’ phosphorylated right oligo</p>

	<p>with a 3' universal amplification tail. To assess fetal fraction, we designed assays against a set of 192 SNP-containing loci on chr1-12, where two middle oligos, differing by one base, were used to query each SNP. SNPs were optimized for minor allele frequency in the HapMap 3 dataset (http://hapmap.ncbi.nlm.nih.gov/).</p> <p>Oligonucleotides were synthesized by IDT and pooled together to create a single multiplexed DANSR assay pool.”</p> <p>[Aria AJOG Publication at 6.]</p> <p>“8mL blood per subject was collected into a Cell-free DNA tube (Streck) and stored at room temperature for up to 3 days. Plasma was isolated from blood via double centrifugation and stored at -20C for up to a year. cfDNA was isolated from plasma using Viral NA DNA purification beads (Dynal), biotinylated, immobilized on MyOne C1 streptavidin beads (Dynal), and annealed with the multiplexed DANSR oligonucleotide pool.</p> <p>[Aria AJOG Publication at 7.]</p> <p>See also above re. claim 1.</p> <p>“This nested case-control study has shown that in pregnancies at high-risk for aneuploidies chromosome-selective sequencing of cfDNA in maternal plasma obtained during the first trimester of pregnancy distinguished all cases of trisomy 21 and 98% of trisomy 18 from euploid pregnancies. The FORTE algorithm combined the risk computed from DANSR with the maternal age-related risks to estimate the patient-specific odds of trisomy versus disomy. In all cases of trisomy 21 the estimated risk for this aneuploidy was >99%, whereas in all euploid pregnancies and in those with trisomy 18 the risk score for trisomy 21 was <0.01%. In the case of trisomy 18 non-invasive testing correctly identified 98% of the cases, where the risk score for this aneuploidy was more than 88%, whereas in all euploid pregnancies and in those with trisomy 21 the risk score for trisomy 18 was less than 0.3%.</p> <p>[AJOG Paper, Ashoor et al. (“Ashoor”) at 10]</p>
<p>22. The method according to claim 21, wherein the non-cellular fraction as used in step (iii) is a plasma fraction.</p>	<p>“8mL blood per subject was collected into a Cell-free DNA tube (Streck) and stored at room temperature for up to 3 days. Plasma was isolated from blood via double centrifugation and stored at -20C for up to a year. cfDNA was isolated from plasma using Viral NA DNA purification beads (Dynal), biotinylated, immobilized on MyOne C1 streptavidin beads (Dynal), and annealed with the multiplexed DANSR oligonucleotide pool.</p> <p>[Aria AJOG Publication at 7.]</p>

<p>24. A method for detecting a paternally inherited nucleic acid on a maternal blood sample, which method comprises:</p> <p>removing all or substantially all nucleated and anucleated cell populations from the blood sample,</p> <p>amplifying a paternally inherited nucleic acid from the remaining fluid and subjecting the amplified nucleic acid to a test for the Paternally inherited fetal nucleic acid.</p>	<p>“Objective</p> <p>To develop a novel biochemical assay and algorithm for the prenatal evaluation of risk for fetal trisomy 21 and 18 using cell-free DNA obtained from maternal blood.”</p> <p>[Aria AJOG Publication, Abstract]</p> <p>“8mL blood per subject was collected into a Cell-free DNA tube (Streck) and stored at room temperature for up to 3 days. Plasma was isolated from blood via double centrifugation and stored at -20C for up to a year.”</p> <p>[Aria AJOG Publication at 7.]</p> <p>“DANSR assay</p> <p>We designed DANSR assays against loci in the human genome as previously described. To assess chromosome proportion, we designed assays against 576 non-polymorphic loci on each of chr18 and chr21, where each assay consisted of three locus specific oligonucleotides: a left oligo with a 5’ universal amplification tail, a 5’ phosphorylated middle oligo, and a 5’ phosphorylated right oligo with a 3’ universal amplification tail. To assess fetal fraction, we designed assays against a set of 192 SNP-containing loci on chr1-12, where two middle oligos, differing by one base, were used to query each SNP. SNPs were optimized for minor allele frequency in the HapMap 3 dataset (http://hapmap.ncbi.nlm.nih.gov/).</p> <p>Oligonucleotides were synthesized by IDT and pooled together to create a single multiplexed DANSR assay pool.”</p> <p>[Aria AJOG Publication at 6.]</p> <p>“8mL blood per subject was collected into a Cell-free DNA tube (Streck) and stored at room temperature for up to 3 days. Plasma was isolated from blood via double centrifugation and stored at -20C for up to a year. cfDNA was isolated from plasma using Viral NA DNA purification beads (Dynal), biotinylated, immobilized on MyOne C1 streptavidin beads (Dynal), and annealed with the multiplexed DANSR oligonucleotide pool. Appropriately hybridized oligonucleotides were catenated with Taq ligase, eluted from the cfDNA, and amplified using universal PCR primers. PCR product from 96 independent samples was pooled and used as template for cluster amplification on a single lane of a TruSeq v2 SR flow slide (Illumina). The slide was processed on an Illumina HiSeq 2000 to produce a 56 base locus-specific sequence and a 7 base sample tag sequence from an average of 1.18 million (M) clusters/sample. Locus specific reads were compared to expected locus sequences. An average of 1.15M (97%) reads had fewer than 3 mismatches with expected locus sequences, resulting in an average of 854 reads/locus/sample.</p> <p>[Aria AJOG Publication at 7.]</p>
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Analysis of non-polymorphic loci for chromosome proportion

Sequence counts were normalized by systematically removing sample and assay biases. Sequence counts follow a log normal distribution, so biases were estimated using median polish on log transformed counts.²⁵⁻²⁷ A chr21 proportion metric was then computed for each sample as the mean of counts for selected chr21 loci divided by the sum of the mean of counts for selected chr21 loci and the mean of counts for all 576 chr18 loci. A chr18 proportion metric was similarly calculated for each sample. A standard Z test of proportions was used to compute Z Statistics,

$$z_j = \frac{p_j - p_0}{\sqrt{\frac{p_0(1-p_0)}{n_j}}}$$

where p_j is the observed proportion for a given chromosome of interest in a given sample j , p_0 is the expected proportion for the given test chromosome calculated as the median p_j , and n_j is the denominator of the proportion metric. ...”

[Aria AJOG Publication at 7-8.]

“Locus selection using training samples

Sequence count data from the training samples were first normalized as described above and previously. These samples were subsequently analyzed to select 384 of the 576 loci on chr21 and chr18 best able to discriminate T21 and T18 from normal samples. The 384 loci on each chromosome exhibiting the greatest residual difference between normal and trisomy samples were identified using Z Statistics derived from individual loci for the test chromosome and all 576 loci for the comparison chromosome.

Analysis of polymorphic loci for fetal fraction

Informative polymorphic loci were defined as loci where fetal alleles differ from maternal alleles. Because DANSR exhibits allele specificities exceeding 99%, informative loci were readily identified when the fetal allele proportion of a locus was measured to be

	<p>between 1 and 20%. A maximum likelihood estimate using the binomial distribution was employed to determine the most likely fetal fraction based upon measurements from several informative loci. The results correlate well ($R^2 > 0.99$) with the weighted average approach presented by Chu and colleagues.” [Aria AJOG Publication at 8-9.]</p> <p>“DANSR enables genotyping of individual polymorphic loci which is not possible using current MPSS approaches. DANSR allowed us to develop an integrated assay to assess polymorphic as well as non-polymorphic loci, thereby permitting simultaneous determination of fetal fraction and chromosome proportion. We used fetal fraction information by imposing a QC requirement that each sample have at least 3% fetal DNA, thereby avoiding low confidence calls arising from low proportions of fetal DNA. In addition, we developed the FORTE algorithm to produce a fetal fraction-dependent risk score indicating the odds of a sample being trisomic versus disomic.” [Aria AJOG Publication at 14.]</p> <p><u>“Importance of fetal fraction</u> A principal determinant of the accuracy of any cfDNA analysis method is the fraction of fetal cfDNA in the sample. The higher the fraction of fetal cfDNA, the greater the difference in the number of cfDNA fragments originating from trisomic versus disomic chromosomes and hence the easier it is to detect trisomy. The FORTE algorithm explicitly accounts for fetal fraction in calculating trisomy risk.” [Aria AJOG Publication at 16.]</p>
25. A method for performing a prenatal diagnosis on a maternal blood sample, which method comprises	<p>“Objective To develop a novel biochemical assay and algorithm for the prenatal evaluation of risk for fetal trisomy 21 and 18 using cell-free DNA obtained from maternal blood.” [Aria AJOG Publication, Abstract]</p>
obtaining a non-cellular fraction of the blood sample	<p>“8mL blood per subject was collected into a Cell-free DNA tube (Streck) and stored at room temperature for up to 3 days. Plasma was isolated from blood via double centrifugation and stored at -20C for up to a year.” [Aria AJOG Publication at 7.]</p>

<p>amplifying a paternally inherited nucleic acid from the non-cellular fraction</p>	<p>“DANSR assay</p> <p>We designed DANSR assays against loci in the human genome as previously described. To assess chromosome proportion, we designed assays against 576 non-polymorphic loci on each of chr18 and chr21, where each assay consisted of three locus specific oligonucleotides: a left oligo with a 5’ universal amplification tail, a 5’ phosphorylated middle oligo, and a 5’ phosphorylated right oligo with a 3’ universal amplification tail. To assess fetal fraction, we designed assays against a set of 192 SNP-containing loci on chr1-12, where two middle oligos, differing by one base, were used to query each SNP. SNPs were optimized for minor allele frequency in the HapMap 3 dataset (http://hapmap.ncbi.nlm.nih.gov/). Oligonucleotides were synthesized by IDT and pooled together to create a single multiplexed DANSR assay pool.”</p> <p>[Aria AJOG Publication at 6.]</p> <p>“8mL blood per subject was collected into a Cell-free DNA tube (Streck) and stored at room temperature for up to 3 days. Plasma was isolated from blood via double centrifugation and stored at -20C for up to a year. cfDNA was isolated from plasma using Viral NA DNA purification beads (Dynal), biotinylated, immobilized on MyOne C1 streptavidin beads (Dynal), and annealed with the multiplexed DANSR oligonucleotide pool. Appropriately hybridized oligonucleotides were catenated with Taq ligase, eluted from the cfDNA, and amplified using universal PCR primers. PCR product from 96 independent samples was pooled and used as template for cluster amplification on a single lane of a TruSeq v2 SR flow slide (Illumina). The slide was processed on an Illumina HiSeq 2000 to produce a 56 base locus-specific sequence and a 7 base sample tag sequence from an average of 1.18 million (M) clusters/sample. Locus specific reads were compared to expected locus sequences. An average of 1.15M (97%) reads had fewer than 3 mismatches with expected locus sequences, resulting in an average of 854 reads/locus/sample.</p> <p>[Aria AJOG Publication at 7.]</p>
<p>and performing nucleic acid analysis on the amplified nucleic acid to detect paternally inherited fetal nucleic acid.</p>	<p>“DANSR assay</p> <p>We designed DANSR assays against loci in the human genome as previously described. To assess chromosome proportion, we designed assays against 576 non-polymorphic loci on each of chr18 and chr21, where each assay consisted of three locus specific oligonucleotides: a left oligo with a 5’ universal amplification tail, a 5’ phosphorylated middle oligo, and a 5’ phosphorylated right oligo with a 3’ universal amplification tail. To assess fetal fraction, we designed assays against a set of 192 SNP-containing loci on chr1-12,</p>

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[Aria AJOG Publication at 14.]

Importance of fetal fraction

A principal determinant of the accuracy of any cfDNA analysis method is the fraction of fetal cfDNA in the sample. The higher the fraction of fetal cfDNA, the greater the difference in the number of cfDNA fragments originating from trisomic versus disomic chromosomes and hence the easier it is to detect trisomy. The FORTE algorithm explicitly accounts for fetal fraction in calculating trisomy risk.”

[Aria AJOG Publication at 16.]